nature reviews bioengineering

https://doi.org/10.1038/s44222-024-00154-9

Review article

Check for updates

The mechanisms of nanoparticle delivery to solid tumours

Luan N. M. Nguyen[1,2], Wayne Ngo[1,2,3,4], Zachary P. Lin[1,2], Shrey Sindhwani[1,2,5], Presley MacMillan[2,6], Stefan M. Mladjenovic[1,2] & Warren C. W. Chan[1,2,6,7,8]

Abstract

Nanoparticles for the detection and treatment of cancer have suffered from limited clinical translation. A key problem has been the lack of understanding of the mechanisms of nanoparticle delivery to solid tumours. The current delivery mechanism is called the enhanced permeability and retention effect, which states that nanoparticles passively enter the tumour through gaps between endothelial cells and are retained because of poor lymphatic drainage. However, nanoparticles designed according to the enhanced permeability and retention effect have limited delivery to solid tumours. An alternative mechanism proposes that nanoparticles enter the tumour through active endothelial transport processes, are retained in the tumour due to interactions with tumour components and exit the tumour through lymphatic vessels. This mechanism is called the active transport and retention principle. In this Review, we explore the contrasting views of these two mechanisms of nanoparticle delivery to solid tumours, explaining the underlying biological mechanisms and their effect on nanoparticle design for cancer applications. Defining the nanoparticle delivery mechanisms to solid tumours is crucial to the advancement and clinical translation of cancer nanomedicines and to determining how nanoparticles should be engineered for medical use.

Sections

[1]Institute of Biomedical Engineering, University of Toronto, Rosebrugh Building, Toronto, Ontario, Canada. [2]Terrence Donnelly Centre for Cellular and Biomolecular Research, University of Toronto, Toronto, Ontario, Canada. [3]Gladstone Institutes, San Francisco, CA, USA. [4]California Institute for Quantitative Biosciences, University of California Berkeley, Berkeley, CA, USA. [5]Temerty Faculty of Medicine, University of Toronto, Toronto, Ontario, Canada. [6]Department of Chemistry, University of Toronto, Toronto, Ontario, Canada. [7]Department of Chemical Engineering and Applied Chemistry, University of Toronto, Toronto, Ontario, Canada. [8]Department of Material Science and Engineering, University of Toronto, Toronto, Ontario, Canada. e-mail: warren.chan@utoronto.ca

Key points

- Nanoparticles can carry imaging agents and therapeutics for the detection and treatment of cancer and need to be delivered to the tumour at a high enough dose to be medically useful.
- The enhanced permeability and retention (EPR) effect states that nanoparticle delivery to tumours is caused by interendothelial gaps in the vasculature and dysfunctional lymphatic vessels, and has long guided the design of cancer nanomedicines.
- Nanoparticles often fail in clinical trials for cancer treatment, likely due to a lack of mechanistic understanding of the delivery process.
- The EPR effect is insufficient to explain nanoparticle delivery into solid tumours.
- The active transport and retention principle challenges the EPR effect, proposing active nanoparticle entry, retention and exit mechanisms as underlying nanoparticle delivery to solid tumours.

Introduction

Anti-cancer treatments and imaging agents based on small molecules, antibodies, nucleic acids, proteins and gene editing tools often do not exhibit ideal pharmacokinetic and pharmacodynamic properties in their native form. These agents can be toxic, unstable, excreted quickly or lack disease specificity. To protect healthy tissue from such agents and optimize their biodistribution to improve tumour delivery, these agents can be packaged into nanoparticles[1].

Nanoparticles are materials on the nanometre length scale and are engineered by tuning their physicochemical properties and conjugating or encapsulating them with functional moieties. This engineering of nanoparticle carriers began in the 1960s, with the synthesis of spherical lipid bilayers termed liposomes. At the time, these nanoparticles had a polydisperse size and could only encapsulate ions and small molecules[2,3]. In contrast to these early nanoparticles, nanoparticles today can be synthesized with metals, polymers, proteins and other materials to a precise size[1]. They can be conjugated with ligands to target specific cells in the body[4–6] and can encapsulate payloads such as DNA[7–9], RNA[10–12], proteins[13–15] and small molecules[16–18]. Nanoparticles can be programmed to release their payload in response to different biological and external stimuli, including pH[19,20], temperature[21,22] and electromagnetic fields[23,24]. Once the nanoparticle system has been chemically designed, they are administered into animal models or human patients, where they circulate throughout the body and accumulate inside the tumour. In the tumour, nanoparticles can generate imaging signals for cancer detection or release drug payloads for localized therapy.

The enhanced permeability and retention (EPR) effect has long served as the mechanism of nanoparticle delivery to tumours. The EPR effect states that nanoparticles passively enter the tumour through interendothelial gaps along the blood vessel wall and that dysfunctional lymphatics within the tumour limit the nanoparticles from exiting[25–28]. The absence of an exit route causes the nanoparticles to be retained inside the tumour. The EPR effect suggests that the main nanoparticle design parameters to consider for tumour delivery are size and circulation time[29]. Nanoparticles should be designed with sizes smaller than the size of interendothelial gaps to accumulate inside the tumour, with sufficient circulation time for this accumulation to occur.

However, relying on the EPR effect to guide cancer nanomedicine design has had limited clinical success. To date, only 15 cancer nanomedicines have been approved for clinical use globally[30]. The large majority (86%) of cancer nanomedicines fail in phase III clinical trials owing to a lack of therapeutic efficacy[30], and no targeted nanoparticles for cancer treatment are currently approved. The poor clinical translation of cancer nanomedicines has sparked debate about the relevance of the EPR effect in nanoparticle delivery to tumours[31–35].

In this Review, we discuss the mechanisms of nanoparticle delivery to solid tumours. We explore the history and impact of the EPR effect and introduce an alternative mechanism, called the active transport and retention (ATR) principle (Fig. 1). We also examine the use of the ATR principle to guide the engineering of nanoparticles intended for delivery into solid tumours, highlighting opportunities in cancer nanomedicine design and questions that remain to be addressed from a mechanistic perspective.

Tumours accumulate macromolecules and nanoparticles

It is well documented that macromolecules and nanoparticles accumulate and retain in solid tumours. The earliest reports of this phenomenon date back to the 1900s, when researchers demonstrated the increased accumulation of colloidal dyes in tumours compared to healthy tissues[36,37]. Subsequent studies showed that this tumour accumulation applied to proteins, iron oxide colloids, colloidal carbon, radioactive labels and crystalline substances as well[38–46]. These studies were observational and established the propensity of tumours to accumulate injected particulates. The idea that this phenomenon could be applied to deliver nanoparticles to tumours for therapeutic and diagnostic applications did not emerge until the 1980s.

In 1984, the concepts of EPR were first stated together to explain the preferential accumulation and retention of SMANCS–lipiodol in a tumour[47] (Fig. 1). SMANCS is a protein–polymer conjugate made of the protein neocarzinostatin (NCS) surface-conjugated to a copolymer of styrene and maleic acid (SMA). This conjugate has a mass between 15 and 18 kDa and is soluble in lipophilic solvents such as lipiodol. SMANCS–lipiodol was administered into the hepatic artery of liver tumour-bearing rabbits and after 24 h was found to have a bioactivity of 12.2 and 3.9 μg ml^{-1} in the tumour and healthy liver tissue, respectively, indicating preferential tumour accumulation. After 7 days, SMANCS had a bioactivity of 0.6 and 0.4 μg ml^{-1} in the tumour and healthy liver tissue, respectively, indicating preferential tumour retention. This preferred tumour accumulation was speculated to be caused by the enhanced permeability of angiogenic tumour blood vessels, and the enhanced retention was caused by dysfunctional lymphatic drainage.

In 1986, the concept of enhanced accumulation and retention was generalized to all macromolecules[29] (Fig. 1). The macromolecules used were radiolabelled proteins with masses between 15 and 70 kDa. When these macromolecules were injected into sarcoma-180 tumour-bearing mice, they were shown to take 19–68 h to reach a tumour-to-blood ratio of 5, which provided evidence for the preferential accumulation and retention of macromolecules in tumours. Colourimetric experiments supported this hypothesis, showing that Evans blue bound to albumin progressively turned the tumour more blue than control skin tissue, and that the blue pigment was retained in the tumour for over 3 days whereas the control tissue returned to its original pigment over this same time period. This preferential accumulation and retention

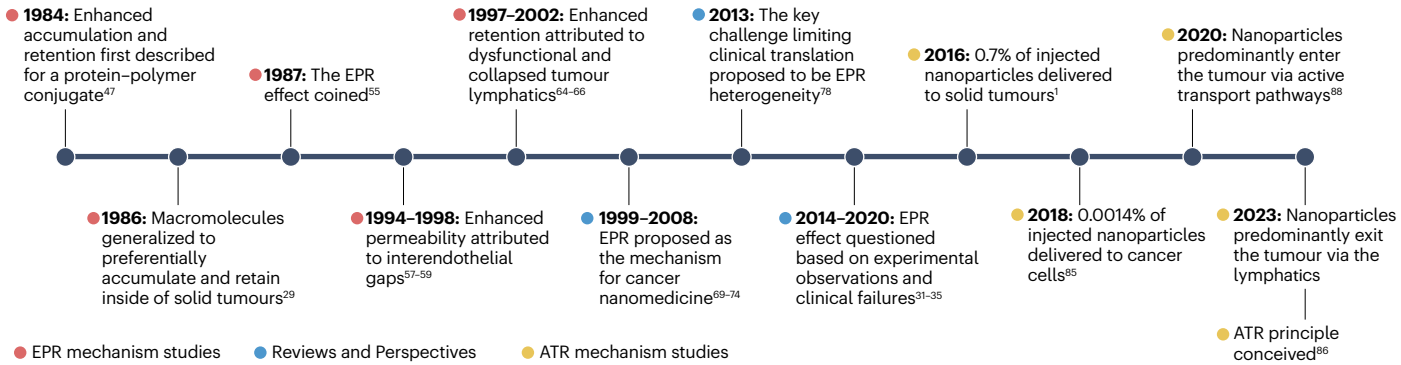

Fig. 1 | Timeline of the mechanisms of nanoparticle delivery to solid tumours. The timeline highlights key studies or a sequence of studies that led to the formulation of the enhanced permeability and retention (EPR) effect[29,47,55,57–59,64–66] (red points) and the active transport and retention (ATR) principle (yellow points)[1,85,86,88] for nanoparticle delivery to solid tumours. Key Review articles and Perspectives are included (blue points)[31–35,69–74,78].

was postulated to result from the permeable blood vasculature and dysfunctional lymphatic system in the tumour but direct mechanistic evidence for these claims was not presented. Two studies published that same year further supported this preferential accumulation and retention of macromolecules. The first study showed that the vascular permeability of 150 kDa dextran was eight times higher in VX2 tumour-bearing rabbits than in healthy tissue using pharmacokinetic data and mathematical modelling[48]. The second study showed that the accumulation of dye-bound proteins was sevenfold higher in Walker 256 carcinoma-bearing rats than in healthy tissues using fluorescent live imaging[49]. Therefore, macromolecules were proposed instead of small molecules for cancer therapy.

In the 1990s, numerous articles were published that used nanoparticles for cancer therapy. Nanoparticles were initially referred to as polymer colloids and protein aggregates at this time[50,51], but this definition has since been expanded to include liposomes, inorganic colloids, macromolecules and other materials in the nanometre length scale[1]. When these nanoparticles were administered to tumour-bearing animals, they were shown to behave similarly to macromolecules. In one example, polyethylene glycol (PEG)-conjugated fullerenes were shown to accumulate more in Meth A fibrosarcoma-bearing mice than in healthy skin and muscle and were then retained in the tumour for at least 2 days[52]. In another example, PEG-polylactic acid nanoparticles were shown to accumulate more in EMT-6 tumour-bearing mice than in healthy skin and were then retained in the tumour for 1 week[53]. Similar observations were made with solid lipid nanoparticles when comparing accumulation and retention in tumours to those in heart and lung tissues[54]. These studies reaffirm that nanoparticles have enhanced tumour accumulation and retention compared with healthy tissues.

Following the evidence over the past 100 years, it is clear that macromolecules and nanoparticles preferentially accumulate and retain in tumours. What remains debated are the fundamental mechanisms underlying this behaviour. Understanding these mechanisms is crucial to guiding the engineering of agents for optimal medical use in human patients with cancer.

EPR effect

The EPR effect was first coined in 1987 (ref. 55), and it was originally only a phenomenological theory. It took one more decade before the biological mechanisms that underlie this theory were uncovered. The EPR effect states that nanoparticles enter the tumour through gaps between blood vessel endothelial cells and are retained inside the tumour because the exit route is blocked by dysfunctional lymphatic vessels (Fig. 2). Table 1 shows the nanoparticles that exhibit the EPR effect.

Mechanism of nanoparticle entry into solid tumours

The EPR effect posits that nanoparticles enter the tumour through gaps between endothelial cells[25–28]. This entry mechanism was established following a series of publications between 1987 and 1998 (Fig. 1). Tumour blood vessel permeability was first hypothesized to be caused by gaps between tumour endothelial cells[56], and this hypothesis was later tested using 90-nm liposomes[57]. These liposomes were shown to accumulate in the LS174T tumours in mice, and this accumulation was attributed to interendothelial gaps because the liposomes "cannot be easily engulfed then shuttled by vesicles or move freely through vesicular channels or vesiculo-vacuolar organelle[s]"[57]. This liposome accumulation experiment was subsequently repeated with larger-sized liposomes and multiple tumour types[58,59]. Liposomes as large as 380 nm were shown to accumulate in HCaI, LS174T, ST-8, ST-12 and MCaIV tumours in mice, whereas liposomes larger than 780 nm were excluded from most tumours. This result suggests that the interendothelial gaps were 380–780 nm wide. Circular structures (presumed to be liposomes) were observed between interendothelial gaps with electron microscopy, which further supported this entry mechanism. These studies led to the conclusion that the mechanism of nanoparticle entry into the tumour was through gaps between endothelial cells, providing the basis for the 'enhanced permeability' part of the EPR effect.

Mechanism of nanoparticle exit from solid tumours

The EPR effect suggests that nanoparticle exit from solid tumours is lacking[60], impaired[61], dysfunctional[62] or defective[63] because the lumen of tumour lymphatic vessels is collapsed and too small to transport nanoparticles[25–28]. This mechanism of nanoparticle exit was established between 1997 and 2002 (Fig. 1). Using mathematical models to calculate the mechanical forces in tumour spheroids[64], it was first shown that cancer cells exhibit mechanical forces large enough to collapse lymphatic vessels. In a subsequent experimental study, the lymphatic tracer ferritin was shown to not label the lymphatics inside murine FSaII tumours when injected into the dermis upstream of the tumour[65]. Since the ferritin is transported through the lymphatics system and would

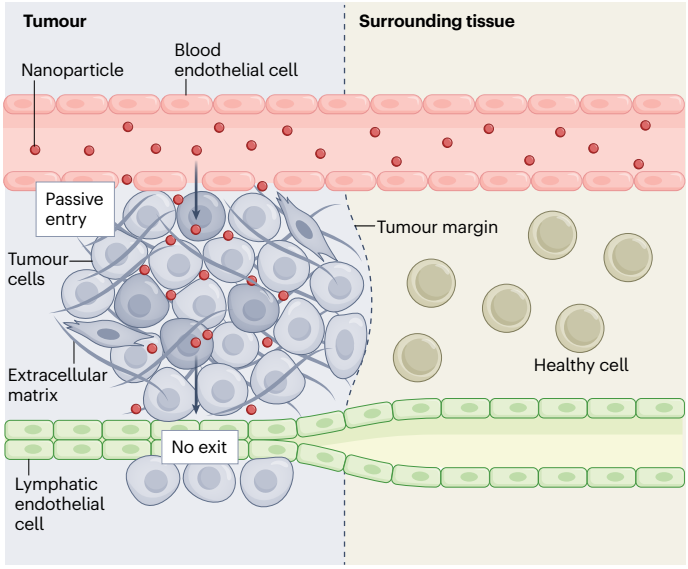

Fig. 2 | EPR mechanism of nanoparticle delivery. The enhanced permeability and retention (EPR) mechanism postulates that nanoparticles enter the tumour through gaps in the tumour blood vessel wall. Once nanoparticles are inside the tumour, the EPR effect suggests that nanoparticles are unable to exit owing to the collapse of the tumour lymphatics. The combination of nanoparticle entry and the absence of exit results in nanoparticle retention inside the tumour.

presumably flow into the tumour lymphatics, the absence of ferritin in the tumour was thought to indicate non-functional lymphatics. This ferritin experiment was then repeated with direct injections into B16F10 tumours[66]. Immunohistochemistry on these tumours revealed that the lymphatic vessels appear as linear streaks without an observable lumen, leading to the conclusion that the vessels were 'collapsed'. The ferritin did not colocalize with these tumour lymphatic vessels, leading to the conclusion that these vessels are dysfunctional. Although a causative relationship between vessel collapse and dysfunction was not shown, and that these studies did not include nanoparticles, these findings led to the idea that collapsed or compressed lymphatic vessels cause dysfunctional lymphatic drainage, which blocks the fluid flow and prevents nanoparticle exit from the tumour[27,28]. This idea provided the basis for the 'poor lymphatic drainage' part of the EPR effect.

Mechanism of nanoparticle retention in solid tumours

According to the EPR effect, nanoparticle retention in solid tumours results from 'enhanced permeability' and impaired nanoparticle exit[25–28] (Fig. 1). This concept was first proposed in 1986 and was the result of four key vasculature characteristics: hypervasculature, enhanced vascular permeability caused by a permeability factor, and minimal recovery of macromolecules via blood vessels and lymphatics[29]. These vascular characteristics are considered to be part of the hallmarks of cancer[67,68]. The tumour hypervasculature and enhanced vascular permeability enable more nanoparticles to reach and enter the tumour space, while the lack of recovery (that is, removal of nanoparticles) by blood vessels and lymphatics limit the nanoparticles from leaving the tumour. As nanoparticles enter the tumour and have limited exit, they would be retained inside the tumour. This mechanism of nanoparticle retention in solid tumours became the 'enhanced retention' portion of the EPR effect.

Successes and limitations of the EPR effect

In the late 1990s and early 2000s, the EPR effect and its impact on cancer nanomedicine was highly praised in numerous articles[69–74] (Fig. 1). The EPR effect was described as "a universal phenomenon in most solid tumours"[73] and "a universal gateway for the selective delivery of macromolecular anticancer medicines"[69]. The EPR effect provided justification to develop nanoparticles for better cancer therapeutics or detection, which was leveraged by academia and industry. In academia, the US National Cancer Institute established the Alliance for Nanotechnology in Cancer programme in 2004, which primarily funded research projects and centres. They collectively received US$ 165 million in initial funding, US$ 500 million in federal grants, and over US$ 1.48 billion in private financing over 15 years, and resulted in over 3,400 publications on nanomedicine for cancer treatment[35,75–77]. In industry, many start-up companies were established to develop nanomedicines. In 2000, Merrimack Pharmaceuticals was formed and has raised over US$ 2 billion in funding to date. They developed numerous cancer nanomedicine candidates, such as the antibody-targeted liposomes MM-302 and MM-310 (ref. 30). In 2007, BIND Therapeutics was formed and raised US$ 914 million during its lifetime. They focused on developing polymer micelles such as BIND-014, a prostate-specific membrane antigen-targeting block copolymer nanoparticle containing docetaxel[30]. These efforts by academia and industry resulted in FDA and/or EMA approvals of numerous nanoparticle cancer therapeutics, such as the liposome nanoparticle Doxil for breast cancer, ovarian cancer, and multiple myeloma, and the albumin nanoparticle Abraxane for breast cancer, pancreatic adenocarcinoma, and non-small cell lung cancer[30].

Yet, despite these successes, most nanomedicines struggle to be approved for clinical use because they lack efficacy in humans. It has been suggested that one cause of this limited efficacy may be tumour heterogeneity or variable pathophysiology between tumour models, animals and human patients[78–81] (Fig. 1). Tumour heterogeneity and its effect on nanoparticle tumour accumulation have been described in several studies. For example, nanoparticle accumulation has been shown to vary as a function of nanoparticle dose and time post-nanoparticle injection using a whole-mouse imaging technique[82]. Nanoparticle accumulation has also been shown to depend on variable blood vessel and macrophage distributions in U87 xenograft mouse models using a computational modelling approach[83]. These studies show that tumour heterogeneity affects nanoparticle tumour accumulation, but before a complete description can emerge, tumour heterogeneity should be physiologically defined and classified. By defining and classifying tumour heterogeneity, nanoparticle performance could be related to a specific tumour physiology, which can be used to identify or stratify patients that may benefit from nanoparticle therapy. However, defining tumour heterogeneity requires correct delivery mechanisms because they provide the key parameters for classifying tumour heterogeneity in the context of nanoparticle delivery.

Nanoparticles need to be delivered to tumours to produce a therapeutic effect in humans. In 2016, we conducted a meta-analysis on nanoparticle designs published between 2005 and 2015 in studies that report the nanoparticle amount in the tumour at three or more time points[1]. We calculated the tumour delivery efficiency of these nanoparticles and found that, although there were more nanoparticles in tumours than in healthy non-reticuloendothelial tissues, only a median of 0.7% (roughly 7 in 1,000) of the injected nanoparticle dose was delivered to tumours across all analysed nanoparticles and tumour models. Strikingly, this 0.7% median did not increase significantly between 2005

and 2015, showing that tumour delivery was not improving over time despite new nanoparticle designs. Repeating this analysis for individual nanomaterial types or tumour models resulted in the same trend. A recent analysis using a physiological-based modelling approach arrived at a similar conclusion, showing that a median of 0.76% and 0.35% of the injected dose of different nanoparticle designs was delivered to the tumour at 24 and 168 h post-administration, respectively[84]. The downstream consequence of a 0.7% delivery efficiency is that an even smaller amount of nanoparticles are available to cancer cells. For example, only 0.0014% of injected gold nanoparticles are delivered to SKOV3 cancer cells in mice[85] (Fig. 1), indicating that they do not accumulate in tumours or cancer cells at high concentrations. These studies suggest that mechanisms in addition or alternative to the EPR effect must be investigated and leveraged.

ATR principle

The active transport and retention (ATR) principle was proposed in 2023 based on our studies and previous investigations by other researchers[86]. The ATR principle states that nanoparticles enter the tumour through active processes involving endothelial cells, are retained due to interactions with tumour cellular and acellular components, and exit through the lymphatics within and around the tumour (Fig. 3 and Table 1).

Mechanism of nanoparticle entry into solid tumours

The ATR principle suggests that the dominant mechanism of nanoparticle entry into solid tumours is through an active transport process. Active processes require energy input (from cells) to transfer nanoparticles from the blood vessel into the tumour. These active processes require the structure of the endothelial cell to change upon spending energy and include mechanisms such as transcytosis, vesiculo-vacuolar organelles and migrating cell effects. Transcytosis pathways include multiple mechanisms, such as phagocytosis, macropinocytosis, caveolar-mediated and clathrin-mediated endocytosis, and caveolar-independent and clathrin-independent mechanisms[87].

Evidence for active transport being the dominant entry mechanism was developed in a study investigating the entry of 15-nm, 50-nm and 100-nm gold nanoparticles into tumours[88] (Fig. 1). Using a mouse model named Zombie, the passive transport of nanoparticles into tumours was shown to only account for 3–25% of tumour accumulation depending on the nanoparticle design. Electron microscopy and mathematical modelling corroborated this conclusion, revealing that the gap density of 500 mm^{-2} could only explain 2.5% of nanoparticle accumulation. Importantly, the blood vasculature in patient-derived human tumours did not have gaps between endothelial cells, suggesting that active processes likely mediate nanoparticle accumulation in humans. Gold nanoparticles were found within the vesicles of blood endothelial cells, implying that transcytosis mechanisms are the dominant active transport pathway for nanoparticles. Using transcriptomics, it was further shown that nanoparticle transcytosis is mediated by a subset of tumour endothelial cells, termed "nanoparticle transport endothelial cells"[89].

In addition to gold nanoparticles, this transcytosis mechanism has been shown for numerous nanoparticle designs. Cationic liposomes (non-PEGylated) 70-nm in size undergo transcytosis in RIP-Tag2 mouse models[90], and 50-nm colloidal carbon undergoes transcytosis in guinea pig subcutaneous cholangiocarcinomas, Lewis lung and TA3/St tumours in mice[91]. The clinically approved formulation Abraxane, which has a size range of 10–130 nm, enters the tumour via transcytosis using the gp60 receptor[92].

Transcytosis is not the only active process to transport nanoparticles. Vesiculo-vacuolar organelles can also transport gold nanoparticle-conjugated albumin and 65-nm liposome–silica hybrid

Table 1 | Evidence of the EPR effect and ATR principle

Delivery mechanism	Transport process	Biological mechanism	Applicable nanoparticles	Refs.
EPR	Entry	Gaps	Liposomes	59
	Exit	Dysfunctional lymphatics[a]	No data	No data
	Retention	Conjecture[b]	No data	No data
ATR	Entry	Transcytosis	Gold nanoparticles, colloidal carbon, cationic liposomes, abraxane	88,90–92
		Vesiculo-vacuolar organelle	Gold nanoparticles, lipid-coated silica nanoparticles	93,94
		Migrating cell effect	Liposomes	96
	Exit	Intratumoural lymphatics	Gold nanoparticles, silica nanoparticles, liposomes, poly(amidoamine) dendrimers	86,100
		Peritumoural lymphatics	Gold nanoparticles, silica nanoparticles, liposomes	86
		Lymphatics unspecified	Quantum dots, single-walled carbon nanotubes, Tc99m colloids, poly(amidoamine) dendrimers, PDPA-TMR polymeric micelles, liposomes	97–99,101–103
		Blood vessel	Gold nanoparticles	86
	Retention	Cellular	Silica nanoparticles, polystyrene nanoparticles, gold nanoparticles, polymeric micelles, PLGA nanoparticles	85,86,105–107
		Acellular[c]	Gold nanoparticles, polystyrene nanoparticles	85,86,119,121

Nanoparticles are only included when direct evidence is presented for a given mechanism, for example, electron microscopy imaging to show nanoparticles transporting through interendothelial gaps or fluorescence imaging of nanoparticle co-localization with a transcytosis marker. Nanoparticles that are engineered to induce a particular mechanism are excluded. Macromolecules, such as dextran, are excluded because they are not traditionally considered nanoparticles. EPR, enhanced permeability and retention; ATR, active transport and retention; PDPA-TMR, poly(ethylene oxide)-b-poly[2-(diisopropylamino)ethyl methacrylate-co-2-aminoethyl methacrylate hydrochloride]-tetramethyl rhodamine; PLGA, poly(lactic-co-glycolic acid). [a]Studies on dysfunctional lymphatics did not use nanoparticles. [b]Enhanced retention was conjectured to be a result of increased nanoparticle tumour entry and limited nanoparticle exit; direct evidence was not presented for nanoparticles. [c]Acellular mechanisms of nanoparticle retention include ex vivo matrix scaffolds.

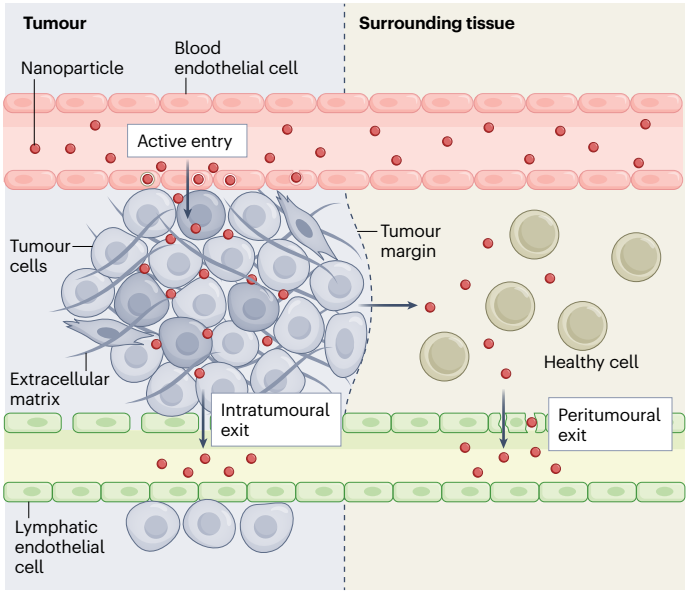

Fig. 3 | ATR mechanism of nanoparticle delivery. The active transport and retention (ATR) mechanism of nanoparticle delivery states that nanoparticles enter the tumour through both active and passive transport mechanisms. Active transport mechanisms include transcytosis mediated by nanoparticle transport endothelial cells, vesicle-vacuolar organelles and migrating cells. These active transport mechanisms are dominant over passive transport, which includes gaps and fenestrations. After entering the tumour, nanoparticles are retained owing to interactions with tumour cellular and acellular components. These tumour components sequester nanoparticles, thus slowing their transport from the entry site to the exit site. Nanoparticles exit the tumour via intratumoural or peritumoural lymphatics. Nanoparticles reach the peritumoural lymphatics by transporting out of the tumour at the tumour margin and accumulating in the tissues surrounding the tumour.

nanoparticles into the tumour[93,94]. In addition, migrating tumour cells and neutrophils create pores in the blood vessel wall for dextrans and 100-nm liposomes, respectively, to enter the tumour[95,96]. These active transport processes appear to be the dominant mechanisms of nanoparticle entry into the tumour.

Mechanism of nanoparticle exit from solid tumours

The ATR principle suggests that the dominant mechanism of nanoparticle exit out of solid tumours is through lymphatic vessels inside (intratumoural) and surrounding the tumour (peritumoural). Evidence for this mechanism was developed in a study investigating the exit of 15-nm, 50-nm and 100-nm gold nanoparticles, 100-nm liposomes, and 100-nm silica nanoparticles from B16F10, 4T1 and MMTV-PyVT solid tumours[86] (Fig. 1). It was shown that 45% of the total accumulated amount of 15-nm gold nanoparticles exit the tumour after 5 days. High-resolution electron microscopy revealed that lymphatic vessels in the tumour are not collapsed and have lumen sizes larger than 286 nm, which is wide enough for nanoparticle transport. Using three-dimensional imaging, histology and electron microscopy, the nanoparticles were shown to exit the tumour via the intratumoural and peritumoural lymphatics. Nanoparticles reach the peritumoural lymphatics by transporting out of the tumour margin and accumulating in the tissues surrounding the tumour, where they are drained by the peritumoural lymphatics.

The dominant lymphatic exit mechanism is dependent on nanoparticle size. Nanoparticles larger than 30 nm predominantly exit the tumour via the intratumoural lymphatics, whereas smaller nanoparticles predominantly exit via the peritumoural lymphatics. Nanoparticles also exit the tumour through blood vessels but this pathway is a minor contributor compared with the lymphatics. Mathematical modelling suggests that the lymphatics remove 6.4% of the injected dose per gram of 15-nm nanoparticles, whereas the blood vasculature only removes 0.3% of the injected dose per gram over 5 days following an intravenous nanoparticle injection. Whole-mouse imaging further showed that 15-nm gold nanoparticles, 100-nm silica nanoparticles and 100-nm liposomes are transported through the lymphatic system after exiting the tumour. The lymphatic system consists of multiple lymph nodes and collecting lymphatic vessels, which connect with the blood vasculature at the thoracic duct or right lymphatic trunks. Gold, silica and liposome nanoparticles transit through the thoracic duct, re-enter the blood vasculature and are re-circulated throughout the body.

This lymphatic exit mechanism may apply to other nanomaterials. For example, quantum dots accumulate in the tumour draining lymph nodes after a direct injection into mouse tumours[97]. Similarly, radioactive Tc99m nanocolloids accumulate in the lymph nodes in human patients with breast cancer[98]. Here, quantum dots and nanocolloids were used to determine the lymphatic drainage basin of the tumour to identify the sentinel lymph nodes for surgical resection. Moreover, polymeric nanomaterials and carbon nanotubes accumulate in the lymphatics after direct injection into mouse tumours[99–103]. These observations support the mechanism of nanoparticle exit via lymphatic mechanisms.

Mechanism of nanoparticle retention in solid tumours

The ATR principle posits that nanoparticles are retained inside the tumour because of their interactions with cellular and acellular components. These interactions trap the nanoparticles inside the tumour (for example, cellular uptake or non-specific binding) as they transport from the entry site to the exit site. Early reports of this trapping effect were made at the beginning of the twentieth century. For example, dyes such as Evans blue and trypan blue were shown to localize at tumour edges and inside stromal cells, suggesting that retention may be caused by binding to or uptake by tumour cells[37,104]. Congo red and sodium iodine localize in the necrotic regions of tumours, suggesting that they bind to acellular tumour components[36]. Therefore, retention of injected particulates may result from binding to cellular and acellular tumour components.

Multiple cell types trap nanoparticles inside the tumour, such as tumour-associated macrophages (TAMs), lymphocytes, monocytes and cancer cells[105,106]. TAMs are considered to be the largest contributor, with one study reporting TAMs to account for over 80% of 500-nm silica nanoparticle uptake after 4 days in OVCAR8 tumour-bearing mice[107] and another study reporting 85% of 55-nm gold nanoparticle uptake in SKOV3 tumour-bearing mice[85]. The amount of nanoparticles that TAMs sequester depends on the physicochemical properties of the nanoparticles[85,105,107,108]: 50 nm appears to be the optimal size for TAM uptake of gold nanoparticles[105], and increasing PEG density on gold nanoparticle surfaces from 0.16 to 0.80 molecules per squared nanometre decreases macrophage sequestration by up to ten times[108]. This sequestration can last for multiple days. TAMs also sequester 500-nm silica, 398-nm poly(lactic-co-glycolic acid) and 798-nm polystyrene nanoparticles for over 4 days in ovarian cancer mouse models[107], and B16F10 tumour cells in mice retain 15-nm gold nanoparticles for over 2 days[86].

Nanoparticles can also interact with the acellular components of a tumour. These components include the tumour matrix, extracellular vesicles, lipoproteins, glycoprotein pools and necrotic cell debris[85,109,110], but the most well-reported tumour component regarding interactions with nanoparticles is the tumour matrix. This matrix includes basement membrane proteins (such as laminin, collagen IV, perlecan and nidogen[111,112]) and interstitial matrix proteins (notably collagen I and III[113–116]). These proteins are arranged in a charged meshwork and spaced 20–160 nm apart, depending on the model and measurement technique[117,118]. In vitro hydrogel models and intravital microscopy on tumours revealed that nanoparticles become trapped within this meshwork as a function of nanoparticle size and charge[119–122]. For example, increasing the size of unmodified polystyrene nanoparticles from 20 nm to 100 nm decreases diffusion displacement by approximately two times in ex vivo MDA-MB-231 xenograft breast cancer tissue from mice[121]. Polystyrene nanoparticles with surface potentials above 7.4 mV and below −38 mV are immobilized in the tumour matrix as shown in hydrogel models[119]. Therefore, tumour matrix components can trap nanoparticles inside the tumour.

Thus, nanoparticle retention is caused by the molecular and physical interactions between nanoparticles and the cellular and acellular components of a tumour. The amount of nanoparticles retained is equal to the amount of nanoparticles trapped in each tumour component and is also equivalent to the amount of nanoparticles that entered the tumour subtracted by the amount that exited. However, the molecular interactions and kinetics (duration of interaction) between nanoparticles and the cellular and acellular tumour structure remain to be investigated to elucidate nanoparticle retention patterns and behaviour in solid tumours.

Comparison of the EPR effect and ATR principle

The EPR effect and ATR principle differ in four key parameters: the description of nanoparticle delivery to solid tumours, the exploitation of tumour biology to improve delivery, the ideal nanoparticle formulation and, most importantly, the scope of the claims. These two mechanisms are not mutually exclusive, as the ATR principle suggests that passive entry mechanisms may occur but have a minor contribution compared to active mechanisms. How these mechanisms are leveraged for improved nanoparticle delivery varies.

While the EPR effect provides guidance only on passive nanoparticle entry by manipulating tumour blood vessel permeability using peptides[123,124], photothermal therapy[125–127] or radioisotope therapy[128,129], the ATR principle provides guidance for the entry, exit and retention processes. For example, nanoparticle entry may be increased by stimulating the active transport of nanoparticles via transcytosis or vesiculo-vacuolar organelles[130]. Nanoparticle exit may be minimized by reducing tumour lymphatic flow using small molecules and antibodies, and nanoparticle retention may be controlled using matrix crosslinking or degrading proteins. The manipulation of the entry, exit and retention processes provides further opportunities for a finer control of nanoparticle delivery.

The EPR effect provides distinct rules for the design of an optimal nanoparticle (that is, a size smaller than interendothelial gaps and long circulation)[25–28]. By contrast, the ATR principle suggests that no ideal nanoparticle exists. The transport process to the diseased target determines the best design. Nanoparticles with the ideal size for entry may have poor retention, nanoparticles optimized for long circulation time may have poor cellular interaction and these processes may differ from one tumour to the next. These variabilities in the complex nano–bio interactions that occur during the delivery journey translate to a large nanoparticle design space. Machine learning tools may aid in designing nanoparticles for transport through varying and dynamic biological barriers.

As opposed to the EPR effect, which is thought to be applicable across tumour types and models[69,73], the ATR principle is not universal in scope. The ATR principle was formulated based on different nanoparticle designs and tumour models, but untested nanoparticles and tumours may prove to be exceptions. For example, blood vessels in Kaposi sarcomas have micrometre-sized endothelial gaps that may cause passive entry processes to dominate[131,132]. Glioblastomas and haemangioblastomas lack intratumoural lymphatics, which may improve nanoparticle retention[133]. Therefore, striving for a universal theory for cancer nanomedicine is bound to fail. Rather, a collection of mechanisms such as those proposed in the ATR principle, will allow nanoparticles to be designed to exploit a particular biological circumstance.

The ATR principle and the cancer nanomedicine journey

The journey of nanoparticles through the body in the context of the ATR principle can be described in the following steps (Fig. 4). Following intravenous administration of nanoparticles, blood proteins adsorb onto the nanoparticle surface forming a protein corona[134–136], which impacts its biological trajectory in vivo[137]. The protein-coated nanoparticles then circulate and interact with different cells and tissues on their way to the tumour. Cell-surface receptors may bind to the protein corona for cellular uptake[108,138,139], with apolipoproteins and complement proteins playing key roles[139]. Cell-surface receptors may also recognize the nanomaterial itself. For example, CD36 mediates the uptake of lipid micelles and may be involved in the uptake of clinically relevant liposome formulations[140], CD206 mediates the uptake of mannose-coated nanoparticles[141], and receptor gp60 mediates the uptake of albumin particles[142]. In addition, non-specific uptake mechanisms such as macropinocytosis may drive cellular uptake[87]. Most injected nanoparticles are sequestered by the liver and spleen, rendering them unavailable for tumour delivery[143–145]. Nanoparticles smaller than 5.5 nm are filtered out of the bloodstream via the kidneys[146].

Nanoparticles that reach the tumour cross the blood endothelium predominantly via active transport processes, including transcytosis, vesicle-vacuolar organelles, migrating cell effects and other processes that remain to be discovered[88,89,93,96]. Transcytosis is mediated by specific endothelial cell phenotypes[89], and intravasating tumour cells and extravasating neutrophils create channels enabling nanoparticles to enter the tumour space[95,96]. A minority of nanoparticles enters the tumour via passive mechanisms such as gaps. Once the nanoparticles are inside the tumour, they transport through the tumour microenvironment and interact with cellular and acellular tumour components. Nanoparticle transport occurs via fluid dynamics[147,148] and cellular-based mechanisms[105,106]. The dominant fluid dynamic transport mechanism is thought to be diffusion because elevated interstitial fluid pressure reduces fluid convection and thus advective nanoparticle transport[148]. Cellular-based mechanisms involve perivascular TAMs, which sequester nanoparticles adjacent to blood vessels and transport them deeper into tumours[105]. TAMs may also act as drug depots, potentially releasing nanoparticles over time[106]. As nanoparticles transport, they interact with tumour cellular and acellular components, which may cause their entrapment inside the tumour, delaying their journey from tumour blood vessels to lymphatic vessels.

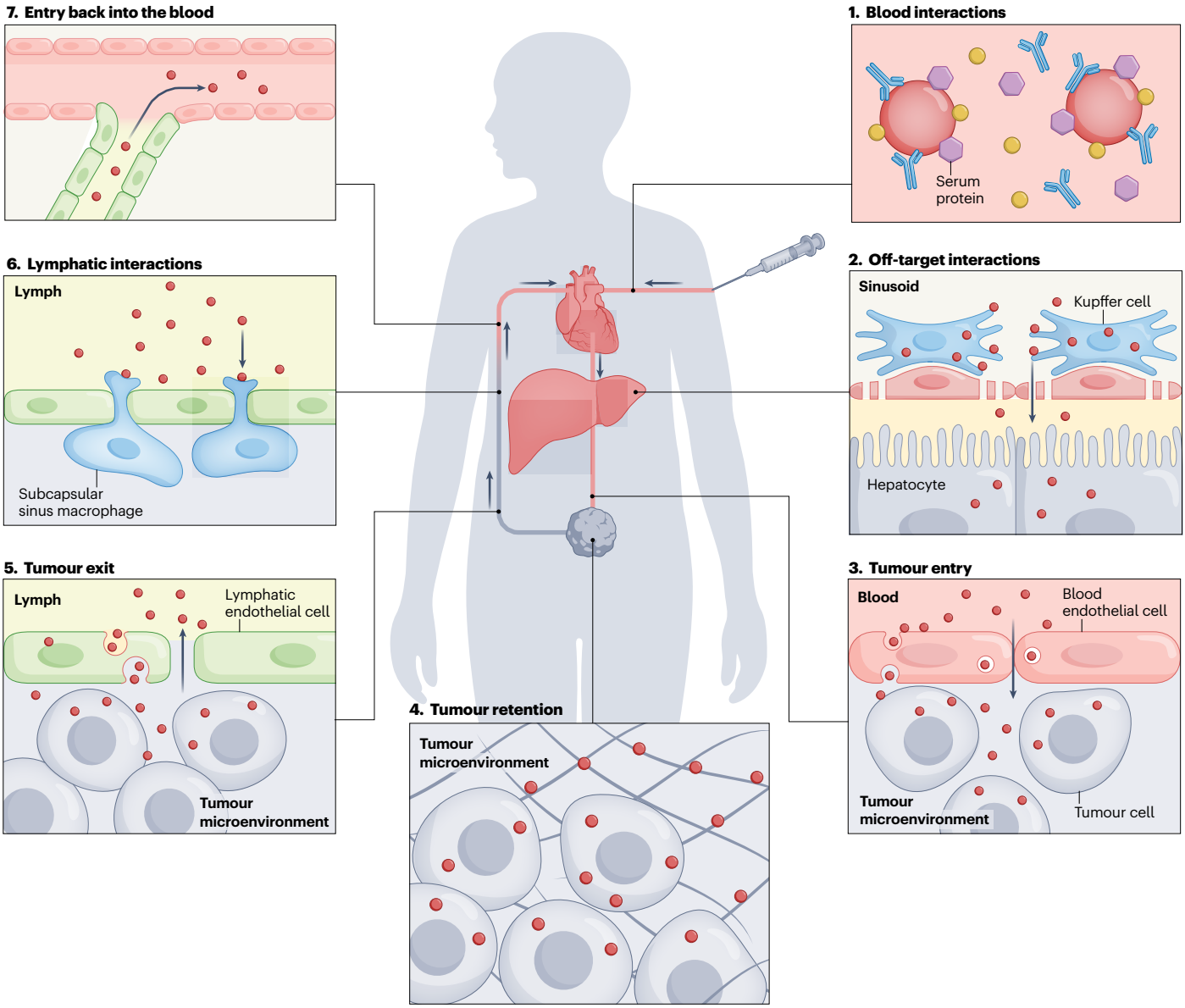

Fig. 4 | The delivery journey of cancer nanomedicine. The journey of nanoparticles once administered into the body. (**1**) Nanoparticles are administered into the blood, where they adsorb blood serum proteins. (**2**) Nanoparticles encounter non-tumour tissues, which sequester the nanoparticles and prevent tumour delivery. In addition to the liver, other organs, such as the spleen and kidneys, clear nanoparticles from the circulation. (**3**) Nanoparticles enter the tumour predominantly via active transport processes such as transcytosis (shown). Other mechanisms include vesiculo-vacuolar organelles and migrating cell effects. Passive transport mechanisms, such as interendothelial gaps, play a minor role. (**4**) Nanoparticles are retained inside the tumour owing to interactions with tumour cells and acellular components. (**5**) Nanoparticles exit the tumour predominantly via the lymphatics. Both lymphatic channels and vesicle-vacuolar organelle mechanisms of exit are shown. Tumour blood vessels contribute a minor role to nanoparticle exit. (**6**) Nanoparticles are transported through the lymphatic circulation, where they encounter immune cells in the lymphatic vessels and lymph nodes, which sequester nanoparticles. (**7**) Nanoparticles are transported back to the blood circulation via the right lymphatic trunks (shown) or thoracic duct. Nanoparticles then repeat this cycle (returning to the first step) until they are cleared from circulation. Nanoparticles, cells and organs are not drawn to scale.

Nanoparticles exit the tumour through intratumoural or peritumoural lymphatics: nanoparticles larger than 30 nm exit the tumour through intratumoural lymphatics, whereas nanoparticles smaller than 30 nm exit through peritumoural lymphatics[86]. Blood vessels contribute a minor amount to nanoparticle exit compared to the lymphatics[86]. Nanoparticles then travel through the lymphatic system, encountering immune cells in the lymph nodes, which may remove them from lymphatic circulation[149–151]. Subcapsular sinus macrophages, follicular dendritic cells and B cells play key roles in sequestering ovalbumin nanoparticles in the lymph node[149,150].

Dendritic cells sequester lipid nanoparticles in the lymph nodes as a function of nanoparticle size and charge[151]. Nanoparticles that escape the lymphatics re-enter the blood circulation at the thoracic duct or right lymphatic trunks. The blood circulation transports the nanoparticles to the heart, where they are circulated throughout the body again[86]. Throughout this repeated journey, the number of nanoparticles available for tumour delivery decreases owing to nanoparticle degradation, elimination, or interactions and sequestration with off-target organs (for example, liver and spleen). Therefore, the efficiency of nanoparticle delivery to tumours is a function of the number of nanoparticles removed from blood circulation by non-tumour cells and tissues, the amount of nanoparticles that enter the tumour, and the amount of nanoparticles that exit the tumour. In essence, the human body provides a series of filter systems that retain, remove or alter nanoparticle transport.

Outlook

Mechanisms can be defined as "the fundamental processes involved in or responsible for an action, reaction, or other natural phenomenon". Mechanisms are fundamentally important because they inform the design of a product to achieve the desired outcome, thereby providing guidelines for technology development. It is thus crucial to determine the correct mechanisms because a wrong mechanism may lead to technologies with disappointing results.

In cancer nanomedicine, the mechanism of nanoparticle delivery guides the design of nanoparticles for the treatment and diagnosis of cancer. The EPR effect has long been the predominant mechanism for nanoparticle tumour delivery, suggesting that nanoparticles smaller than the interendothelial gaps enter the tumour and are subsequently unable to exit despite pharmacokinetic studies showing that the number of nanoparticles in the tumour decreases over time[84,152–160]. Since the mechanism was considered resolved, nanoparticle design focused on tumour cell targeting (with antibodies, aptamers and other moieties) or multifunctionality[26,28,161,162]. Regardless of these diverse designs, many nanoparticle formulations failed in clinical trials due to the inability to demonstrate improved therapeutic efficacy. Factors such as tumour heterogeneity and studies on contrived animal models were thought to play a part in their clinical failures[78–81], leading resarchers to explore patient stratification and animal models more representative of a human tumour. But for these solutions to emerge, it remains key to determine the correct mechanisms of nanoparticle delivery. These mechanisms will identify elements of tumour physiology for the stratification of patients and the biological processes that need to be recapitulated in animal models. Thus, knowledge of the correct delivery

Box 1

Key research questions of the active transport and retention principle

The active transport and retention principle aims to capture the complete interactions of nanoparticles with the tumour environment. However, key details remain to be determined.

Nanoparticle entry mechanisms

The molecular details of nanoparticle entry into solid tumours remain to be investigated, including the receptors involved in 'nanoparticle transport endothelial cell'-mediated transcytosis, the precise molecular machinery involved (for example, caveolin or clathrin[169]) and non-receptor-based transport. In addition, the contributions of different active mechanisms (for example, vesiculo-vacuolar organelles versus transcytosis) need to be determined as well as how these change as a function of nanoparticle design. Understanding these details will enable the design of nanoparticles that have improved tumour entry.

Nanoparticle transport mechanisms

A complete picture of how nanoparticles transport through the tumour microenvironment has yet to be developed. The fluid dynamic mechanisms that transport nanoparticles in the presence of functional lymphatics need to be elucidated as current models do not account for these vessels[147,148]. It also remains unclear whether non-macrophage cell types can transport nanoparticles throughout the tumour. Techniques could be further developed to liberate nanoparticles from these migrating cells. Understanding nanoparticle transport mechanisms may enable a better control of the nanoparticle distribution in the tumour.

Nanoparticle retention mechanisms

The mechanisms of nanoparticle retention need to be further explored. In particular, the mechanisms of nanoparticle uptake by different cells in the tumour to understand whether this process is specific or non-specific (for example, receptor-mediated or through macropinocytosis). This understanding would allow the design of strategies for controlling nanoparticle uptake. In addition, how nanoparticles interact with non-matrix acellular components of the tumour should be investigated and compared with the well-characterized matrix interactions. These studies will inform the design of nanoparticles that are better retained in the tumour.

Nanoparticle exit mechanisms

The mechanisms of nanoparticle exit need to be further investigated, for example, to understand why different lymphatics take up nanoparticles via different mechanisms (for example, intratumoural lymphatics use channels and peritumoural lymphatics use vesiculo-vacuolar organelles)[86]. Moreover, the structural components of the tumour margin need to be identified as the tumour margin blocks large nanoparticles while enabling small nanoparticles passage into the surrounding tissues[86]. Understanding these key details of the exit process will enable the development of methods to keep more nanoparticles inside the tumour.

Box 2

Grand challenges of nanomedicine

Nanomedicines face several grand challenges that limit clinical translation irrespective of the target disease.

Nano–bio interactome

The nano–bio interactome can be defined as the set of interactions between nanoparticles and biological tissues. Biological tissues other than the disease site interact with nanoparticles, thereby limiting therapeutic efficacy. For example, nano–blood protein interactions can define the nanoparticle trajectory in vivo[137]. A deeper understanding of nano–blood protein interactions will enable nanoparticle designs that adsorb a distinct set of blood proteins to improve disease tissue accumulation while minimizing off-target accumulation. Developing an atlas of the nano–bio interactome will further allow the tuning of nanoparticle properties to increase the nanoparticle dose available for delivery.

The target-to-liver ratio

The target-to-liver ratio can be defined as the amount of nanoparticles delivered to the target organ divided by the amount delivered to the liver. Maximizing this ratio is a key challenge for nanomedicines as the liver is responsible for most off-target sequestration of nanoparticles[145]. Understanding the mechanisms of nanoparticle sequestration in the liver would enable the development of techniques that limit liver sequestration through distinct nanoparticle design or by biological manipulation (for example, Kupffer cell priming[170]). Maximizing the target-to-liver ratio will render more nanoparticles available for targeted delivery.

Species scaling rules

Different animal models exhibit different physiological (that is, organ weights) and molecular traits (that is, receptor phenotype and expression). Rodents are most used in cancer nanomedicine research but are not the end user. Optimizing a nanoparticle design in a model organism will likely lead to a species-specific formulation because model organisms may express distinct sets of proteins or physiology, which may affect the mechanisms of nanoparticle distribution. Understanding the molecular and genetic basis for these mechanisms is thus crucial to determining whether a nanoparticle formulation works in the same way in different species.

Patient stratification

Individual patients may respond differently to the same therapeutic treatment. Separating high responders from low responders remains a key challenge in developing and applying nanomedicine. Such differences are ultimately caused by the biological heterogeneity between patients. Tools that predict how patient biology changes their response to nanomedicine must be developed. These tools should be based on biological mechanisms because these relate the biology to nanoparticle function.

mechanisms will aid in reaching the goal of cancer nanomedicine, that is, clinical translation.

Nanoparticle size, shape, surface chemistry and other physicochemical properties influence their interactions with non-tumour tissues, blood vessels, the tumour microenvironment and tumour lymphatics[85,86,88,105,144,145]. The ATR principle provides a mechanistic framework for the design of nanoparticles for specific tumour types and applications. The ATR principle was established using gold, silica and liposome nanoparticles administered to xenograft, syngeneic, spontaneous and patient-derived tumour models. However, it still needs to be further validated and tested across nanoparticle designs and models. Nanoparticles, such as iron oxides, polymeric micelles, quantum dots, and viral-like and lipid nanoparticles, may enter, exit and be retained in the tumour differently and these processes need to be explored. In addition, the ATR principle should be tested for clinically relevant nanoparticles, such as Doxil, and for nanoparticle formulations that failed clinical trials such as BIND-014 and MM-302. These studies will reveal the mechanistic reasons for the clinical outcomes to inform future nanoparticle designs.

Active transport mechanisms have also been investigated for other platforms; for example, the protein P-selectin has been shown to induce active transport across the blood–brain barrier, which can be utilized for brain tumour nanoparticle delivery[163]. Active transport of materials also occurs across endothelial and cancer cells, which can be exploited to improve the accumulation and distribution of polymer–drug conjugates[164,165]. Interestingly, migrating neutrophils also play a role in breaking the basement membrane, which can enhance nanoparticle extravasation[166]. Furthermore, observations such as transient 'bursts' in vascular permeability[167,168] should be investigated to determine their cause and the underlying mechanism (that is, gap formation or active transport processes).

The future of cancer nanomedicine should focus on designing nanoparticles for a specific biology. This focus will require the determination of finer mechanistic details of the ATR principle and of how these mechanisms change as a function of the physicochemical properties of nanoparticles and the tumour model. Many key questions remain, such as how tumour endothelial cells transport nanoparticles into the tumour microenvironment and the contribution of convection, diffusion and cell transport on nanoparticle permeation in the tumour microenvironment (Box 1). In addition, computational models are required that relate the biological details of the tumour to the properties of nanoparticles. Such computational tools can inform chemical and pharmaceutical nanoparticle design and how to exploit tumour carrier localization.

Nanoparticles remain the ideal delivery system for therapeutic and diagnostic agents as they can be engineered with different sizes, shapes, surface chemistries, payloads and targeting agents with high reproducibility, whilst being small enough to transport through blood vessels and access diverse tissues and organs of the body. The principles learned from the interactions of nanoparticles with tumours can also

be adapted to design nanoparticles for other diseases (for example, cystic fibrosis and diabetes) (Box 2). Elucidating nano–bio interactions and organizing these data into principles, mathematical equations and correlations will result in a master blueprint that can guide nanoparticle design for a variety of applications.

Citation diversity statement

We acknowledge that manuscripts by scholars from historically excluded and underrepresented groups have been systematically under-cited. Here, we made every attempt to reference relevant papers in an equitable manner. We considered racial, ethnic, gender and geographical representation during the literature review process.

Published online: 08 February 2024

References

1. Wilhelm, S. et al. Analysis of nanoparticle delivery to tumours. *Nat. Rev. Mater.* **1**, 16014 (2016).
   This article reports that a low amount of nanoparticles (0.7% injected dose) are delivered to solid tumours.
2. Papahadjopoulos, D. & Miller, N. Phospholipid model membranes. I. Structural characteristics of hydrated liquid crystals. *Biochim. Biophys. Acta Biomembr.* **135**, 624–638 (1967).
3. Sessa, G. & Weissmann, G. Phospholipid spherules (liposomes) as a model for biological membranes. *J. Lipid Res.* **9**, 310–318 (1968).
4. Dong, J. et al. EGFR aptamer-conjugated liposome-polycation-DNA complex for targeted delivery of SATB1 small interfering RNA to choriocarcinoma cells. *Biomed. Pharmacother.* **107**, 849–859 (2018).
5. Kim, D., Jeong, Y. Y. & Jon, S. A drug-loaded aptamer–gold nanoparticle bioconjugate for combined CT imaging and therapy of prostate cancer. *ACS Nano* **4**, 3689–3696 (2010).
6. Corsi, F. et al. HER2 expression in breast cancer cells is downregulated upon active targeting by antibody-engineered multifunctional nanoparticles in mice. *ACS Nano* **5**, 6383–6393 (2011).
7. Cohen, H. et al. Sustained delivery and expression of DNA encapsulated in polymeric nanoparticles. *Gene Ther.* **7**, 1896–1905 (2000).
8. Perez, C. et al. Poly(lactic acid)-poly(ethylene glycol) nanoparticles as new carriers for the delivery of plasmid DNA. *J. Control. Release* **75**, 211–224 (2001).
9. Levine, R. M., Pearce, T. R., Adil, M. & Kokkoli, E. Preparation and characterization of liposome-encapsulated plasmid DNA for gene delivery. *Langmuir* **29**, 9208–9215 (2013).
10. Mai, Y. et al. Intranasal delivery of cationic liposome-protamine complex mRNA vaccine elicits effective anti-tumor immunity. *Cell Immunol.* **354**, 104143 (2020).
11. McKay, P. F. et al. Self-amplifying RNA SARS-CoV-2 lipid nanoparticle vaccine candidate induces high neutralizing antibody titers in mice. *Nat. Commun.* **11**, 3523 (2020).
12. Ashley, C. E. et al. Delivery of small interfering RNA by peptide-targeted mesoporous silica nanoparticle-supported lipid bilayers. *ACS Nano* **6**, 2174–2188 (2012).
13. Zinger, A. et al. Collagenase nanoparticles enhance the penetration of drugs into pancreatic tumors. *ACS Nano* **13**, 11008–11021 (2019).
14. Feczkó, T., Tóth, J., Dósa, G. & Gyenis, J. Optimization of protein encapsulation in PLGA nanoparticles. *Chem. Eng. Process Process Intensif.* **50**, 757–765 (2011).
15. Cao, A. et al. A facile method to encapsulate proteins in silica nanoparticles: encapsulated green fluorescent protein as a robust fluorescence probe. *Angew. Chem.* **122**, 3086–3089 (2010).
16. George, T. A. et al. Liposome-encapsulated anthraquinone improves efficacy and safety in triple negative breast cancer. *J. Control. Release* **342**, 31–43 (2022).
17. Ngo, W. et al. DNA-controlled encapsulation of small molecules in protein nanoparticles. *J. Am. Chem. Soc.* **142**, 17938–17943 (2020).
18. Yoo, H. S., Lee, K. H., Oh, J. E. & Park, T. G. In vitro and in vivo anti-tumor activities of nanoparticles based on doxorubicin–PLGA conjugates. *J. Control. Release* **68**, 419–431 (2000).
19. Rayamajhi, S. et al. pH-responsive cationic liposome for endosomal escape mediated drug delivery. *Colloids Surf. B Biointerfaces* **188**, 110804 (2020).
20. Palanikumar, L. et al. pH-responsive high stability polymeric nanoparticles for targeted delivery of anticancer therapeutics. *Commun. Biol.* **3**, 95 (2020).
21. Tai, L.-A. et al. Thermosensitive liposomes entrapping iron oxide nanoparticles for controllable drug release. *Nanotechnology* **20**, 135101 (2009).
22. Sun, J., Yu, Z., Hong, C. & Pan, C. Biocompatible zwitterionic sulfobetaine copolymer-coated mesoporous silica nanoparticles for temperature-responsive drug release. *Macromol. Rapid Commun.* **33**, 811–818 (2012).
23. Vlasova, K. Y. et al. Magnetic liposome design for drug release systems responsive to super-low frequency alternating current magnetic field (AC MF). *J. Colloid Interf. Sci.* **552**, 689–700 (2019).
24. Ge, J., Neofytou, E., Cahill, T. J., Beygui, R. E. & Zare, R. N. Drug release from electric-field-responsive nanoparticles. *ACS Nano* **6**, 227–233 (2012).
25. Chauhan, V. P. & Jain, R. K. Strategies for advancing cancer nanomedicine. *Nat. Mater.* **12**, 958–962 (2013).
26. Peer, D. et al. Nanocarriers as an emerging platform for cancer therapy. *Nat. Nanotechnol.* **2**, 751–760 (2007).
    This review describes the EPR effect, that is, a passive mechanism of nanoparticle accumulation in tumours and retention due to dysfunctional tumour lymphatics.
27. Nakamura, H., Fang, J., Jun, F. & Maeda, H. Development of next-generation macromolecular drugs based on the EPR effect: challenges and pitfalls. *Expert Opin. Drug Deliv.* **12**, 53–64 (2014).
28. Lammers, T., Kiessling, F., Hennink, W. E. & Storm, G. Drug targeting to tumors: principles, pitfalls and (pre-) clinical progress. *J. Control. Release* **161**, 175–187 (2012).
29. Matsumura, Y. & Maeda, H. A new concept for macromolecular therapeutics in cancer chemotherapy: mechanism of tumoritropic accumulation of proteins and the antitumor agent smancs. *Cancer Res.* **46**, 6387–6392 (1986).
    This article established the accumulation and retention of macromolecules in solid tumours, linking these observations to tumour blood vascular leakiness and poor lymphatic drainage, providing the foundation of the EPR effect.
30. He, H., Liu, L., Morin, E. E., Liu, M. & Schwendeman, A. Survey of clinical translation of cancer nanomedicines — lessons learned from successes and failures. *Acc. Chem. Res.* **52**, 2445–2461 (2019).
    This review analyses nanomedicines in clinical trials, and describes the reasons for their success and failure.
31. Nichols, J. W. & Bae, Y. H. EPR: evidence and fallacy. *J. Control. Release* **190**, 451–464 (2014).
32. Danhier, F. To exploit the tumor microenvironment: since the EPR effect fails in the clinic, what is the future of nanomedicine? *J. Control. Release* **244**, 108–121 (2016).
33. Nakamura, Y., Mochida, A., Choyke, P. L. & Kobayashi, H. Nanodrug delivery: is the enhanced permeability and retention effect sufficient for curing cancer? *Bioconjug. Chem.* **27**, 2225–2238 (2016).
34. Park, K. The drug delivery field at the inflection point: time to fight its way out of the egg. *J. Control. Release* **267**, 2–14 (2017).
35. Sun, D., Zhou, S. & Gao, W. What went wrong with anticancer nanomedicine design and how to make it right. *ACS Nano* **14**, 12281–12290 (2020).
36. Weil, R. Chemotherapeutic experiments on rat tumors. *J. Cancer Res.* https://doi.org/10.1158/jcr.1916.95 (1916).
37. Duran-Reynals, F. Studies on the localization of dyes and foreign proteins in normal and malignant tissues. *Am. J. Cancer* **3**, 98–107 (1939).
38. Peterson, H.-I. & Appelgren, K. L. Experimental studies on the uptake and retention of labelled proteins in a rat tumour. *Eur. J. Cancer* **9**, 543–547 (1973).
39. Song, C. W. & Levitt, S. H. Quantitative study of vascularity in Walker carcinoma 256. *Cancer Res.* **31**, 587–589 (1971).
40. Wright, R. L. Vascular permeability in experimental brain tumors. *Angiology* **18**, 69–75 (1967).
41. Gordon, R. T., Hines, J. R. & Gordon, D. Intracellular hyperthermia a biophysical approach to cancer treatment via intracellular temperature and biophysical alterations. *Med. Hypotheses* **5**, 83–102 (1979).
42. Larson, S. M. Mechanisms of localization of gallium-67 in tumors. *Semin. Nucl. Med.* **8**, 193–203 (1978).
43. Som, P. et al. ^{97}Ru-transferrin uptake in tumor and abscess. *Eur. J. Nucl. Med.* **8**, 491–494 (1983).
44. O'Connor, S. W. & Bale, W. F. Accessibility of circulating immunoglobulin G to the extravascular compartment of solid rat tumors. *Cancer Res.* **44**, 3719–3723 (1984).
45. Dvorak, H. F., Harvey, V. S. & McDonagh, J. Quantitation of fibrinogen influx and fibrin deposition and turnover in line 1 and line 10 guinea pig carcinomas. *Cancer Res.* **44**, 3348–3354 (1984).
46. L'Abbé, M. R., Fischer, P. W. F., Trick, K. D. & Chavez, E. R. Effect of dietary selenium and tumor status on the retention of ^{75}Se by tissues and mammary tumors of DMBA-treated rats. *Biol. Trace Elem. Res.* **20**, 179–196 (1989).
47. Iwai, K., Maeda, H. & Konno, T. Use of oily contrast medium for selective drug targeting to tumor: enhanced therapeutic effect and X-ray image. *Cancer Res.* **44**, 2115–2121 (1984).
48. Gerlowski, L. E. & Jain, R. K. Microvascular permeability of normal and neoplastic tissues. *Microvasc. Res.* **31**, 288–305 (1986).
49. Heuser, L. S. & Miller, F. N. Differential macromolecular leakage from the vasculature of tumors. *Cancer* **57**, 461–464 (1986).
50. Kreuter, J. Nanoparticle-based dmg delivery systems. *J. Control. Release* **16**, 169–176 (1991).
51. Oppenheim, R. C. Solid colloidal drug delivery systems: nanoparticles. *Int. J. Pharm.* **8**, 217–234 (1981).
52. Tabata, Y., Murakami, Y. & Ikada, Y. Photodynamic effect of polyethylene glycol-modified fullerene on tumor. *Jpn. J. Cancer Res.* **88**, 1108–1116 (1997).
53. Allemann, E. et al. PEG-coated poly(lactic acid) nanoparticles for the delivery of hexadecafluoro zinc phthalocyanine to EMT-6 mouse mammary tumours. *J. Pharm. Pharmacol.* **47**, 382–387 (1995).
54. Hodoshima, N. et al. Lipid nanoparticles for delivering antitumor drugs. *Int. J. Pharm.* **146**, 81–92 (1997).
55. Matsumura, Y., Oda, T. & Maeda, H. General mechanism of intratumor accumulation of macromolecules: advantage of macromolecular therapeutics. *Gan Kagaku Ryoho Cancer Chemother.* **14**, 821–829 (1987).

56. Jain, R. K. Transport of molecules across tumor vasculature. *Cancer Metastasis Rev.* **6**, 559–593 (1987).
57. Yuan, F. et al. Microvascular permeability and interstitial penetration of sterically stabilized (stealth) liposomes in a human tumor xenograft. *Cancer Res.* **54**, 3352–3356 (1994).
58. Yuan, F. et al. Vascular permeability in a human tumor xenograft: molecular size dependence and cutoff size. *Cancer Res.* **55**, 3752–3756 (1995).
59. Hobbs, S. K. et al. Regulation of transport pathways in tumor vessels: role of tumor type and microenvironment. *Proc. Natl Acad. Sci.* **95**, 4607–4612 (1998).
This article presents electron microscopy images of nanoparticles entering the tumour via interendothelial gaps, suggesting a mechanism of enhanced permeability of the EPR effect.
60. Zhang, Y. et al. Strategies to improve tumor penetration of nanomedicines through nanoparticle design. *Wiley Interdiscip. Rev. Nanomed. Nanobiotechnol.* **11**, e1519 (2019).
61. Bazak, R., Houri, M., Achy, S. E., Hussein, W. & Refaat, T. Passive targeting of nanoparticles to cancer: a comprehensive review of the literature. *Mol. Clin. Oncol.* **2**, 904–908 (2014).
62. Wang, M. & Thanou, M. Targeting nanoparticles to cancer. *Pharmacol. Res.* **62**, 90–99 (2010).
63. Kalyane, D. et al. Employment of enhanced permeability and retention effect (EPR): Nanoparticle-based precision tools for targeting of therapeutic and diagnostic agent in cancer. *Mater. Sci. Eng. C* **98**, 1252–1276 (2019).
64. Helmlinger, G., Netti, P. A., Lichtenbeld, H. C., Melder, R. J. & Jain, R. K. Solid stress inhibits the growth of multicellular tumor spheroids. *Nat. Biotechnol.* **15**, 778–783 (1997).
65. Leu, A. J., Berk, D. A., Lymboussaki, A., Alitalo, K. & Jain, R. K. Absence of functional lymphatics within a murine sarcoma: a molecular and functional evaluation. *Cancer Res.* **60**, 4324–4327 (2000).
66. Padera, T. P. et al. Lymphatic metastasis in the absence of functional intratumor lymphatics. *Science* **296**, 1883–1886 (2002).
This article presents evidence that tumour lymphatics are dysfunctional and collapsed, suggesting a mechanism of poor lymphatic drainage of the EPR effect.
67. Hanahan, D. & Weinberg, R. A. The hallmarks of cancer. *Cell* **100**, 57–70 (2000).
68. Hanahan, D. & Weinberg, R. A. Hallmarks of cancer: the next generation. *Cell* **144**, 646–674 (2011).
69. Duncan, R. Polymer conjugates for tumour targeting and intracytoplasmic delivery. The EPR effect as a common gateway? *Pharm. Sci. Technol. Today* **2**, 441–449 (1999).
70. Duncan, R. The dawning era of polymer therapeutics. *Nat. Rev. Drug Discov.* **2**, 347–360 (2003).
71. Ferrari, M. Cancer nanotechnology: opportunities and challenges. *Nat. Rev. Cancer* **5**, 161–171 (2005).
72. Duncan, R. Polymer conjugates as anticancer nanomedicines. *Nat. Rev. Cancer* **6**, 688–701 (2006).
73. Greish, K. Enhanced permeability and retention of macromolecular drugs in solid tumors: a royal gate for targeted anticancer nanomedicines. *J. Drug Target.* **15**, 457–464 (2008).
74. Maeda, H., Wu, J., Sawa, T., Matsumura, Y. & Hori, K. Tumor vascular permeability and the EPR effect in macromolecular therapeutics: a review. *J. Control. Release* **65**, 271–284 (2000).
75. Grodzinski, P., Liu, C. H., Hartshorn, C. M., Morris, S. A. & Russell, L. M. NCI alliance for nanotechnology in cancer — from academic research to clinical interventions. *Biomed. Microdevices* **21**, 32 (2019).
76. Ptak, K., Farrell, D., Panaro, N. J., Grodzinski, P. & Barker, A. D. The NCI alliance for nanotechnology in cancer: achievement and path forward. *Wiley Interdiscip. Rev. Nanomed. Nanobiotechnol.* **2**, 450–460 (2010).
77. Grodzinski, P. NCI cancer nanotechnology centers of excellence (CCNEs) — a full story to set the record straight. *J. Control. Release* **309**, 341–342 (2019).
78. Prabhakar, U. et al. Challenges and key considerations of the enhanced permeability and retention effect for nanomedicine drug delivery in oncology. *Cancer Res.* **73**, 2412–2417 (2013).
79. Fang, J., Islam, W. & Maeda, H. Exploiting the dynamics of the EPR effect and strategies to improve the therapeutic effects of nanomedicines by using EPR effect enhancers. *Adv. Drug Deliv. Rev.* **157**, 142–160 (2020).
80. Golombek, S. K. et al. Tumor targeting via EPR: strategies to enhance patient responses. *Adv. Drug Deliv. Rev.* **130**, 17–38 (2018).
81. van der Meel, R. et al. Smart cancer nanomedicine.*Nat. Nanotechnol.* **14**, 1007–1017 (2019).
82. Biancacci, I. et al. Monitoring EPR effect dynamics during nanotaxane treatment with theranostic polymeric micelles. *Adv. Sci.* **9**, 2103745 (2022).
83. MacMillan, P. et al. Toward predicting nanoparticle distribution in heterogeneous tumor tissues. *Nano Lett.* **23**, 7197–7205 (2023).
84. Cheng, Y.-H., He, C., Riviere, J. E., Monteiro-Riviere, N. A. & Lin, Z. Meta-analysis of nanoparticle delivery to tumors using a physiologically based pharmacokinetic modeling and simulation approach. *ACS Nano* **14**, 3075–3095 (2020).
85. Dai, Q. et al. Quantifying the ligand-coated nanoparticle delivery to cancer cells in solid tumors. *ACS Nano* **12**, 8423–8435 (2018).
This article established that only 0.0014% of injected nanoparticles are delivered to cancer cells.
86. Nguyen, L. N. M. et al. The exit of nanoparticles from solid tumours.*Nat. Mater.* **22**, 1261–1272 (2023).
This article reports that nanoparticles exit the tumour through the lymphatics in and around the tumour, and proposes the ATR principle as an alternative mechanism of nanoparticle delivery.
87. Petros, R. A. & DeSimone, J. M. Strategies in the design of nanoparticles for therapeutic applications. *Nat. Rev. Drug Discov.* **9**, 615–627 (2010).
88. Sindhwani, S. et al. The entry of nanoparticles into solid tumours. *Nat. Mater.* **19**, 566–575 (2020).
This article reports that active transport processes of nanoparticle entry are dominant over passive transport.
89. Kingston, B. R. et al. Specific endothelial cells govern nanoparticle entry into solid tumors. *ACS Nano* **15**, 14080–14094 (2021).
90. Thurston, G. et al. Cationic liposomes target angiogenic endothelial cells in tumors and chronic inflammation in mice. *J. Clin. Invest.* **101**, 1401–1413 (1998).
91. Feng, D., Nagy, J. A., Dvorak, H. F. & Dvorak, A. M. Ultrastructural studies define soluble macromolecular, particulate, and cellular transendothelial cell pathways in venules, lymphatic vessels, and tumor-associated microvessels in man and animals. *Microsc. Res. Tech.* **57**, 289–326 (2002).
92. Desai, N. et al. Increased antitumor activity, intratumor paclitaxel concentrations, and endothelial cell transport of cremophor-free, albumin-bound paclitaxel, ABI-007, compared with cremophor-based paclitaxel. *Clin. Cancer Res.* **12**, 1317–1324 (2006).
93. Kohn, S., Nagy, J. A., Dvorak, H. F. & Dvorak, A. M. Pathways of macromolecular tracer transport across venules and small veins. Structural basis for the hyperpermeability of tumor blood vessels. *Lab. Invest.* **67**, 596–607 (1992).
94. Liu, X. et al. Tumor-penetrating peptide enhances transcytosis of silicasome-based chemotherapy for pancreatic cancer. *J. Clin. Investig.* **127**, 2007–2018 (2017).
95. Harney, A. S. et al. Real-time imaging reveals local, transient vascular permeability, and tumor cell intravasation stimulated by $TIE2^{hi}$ macrophage-derived VEGFA. *Cancer Discov.* **5**, 932–943 (2015).
96. Naumenko, V. A. et al. Extravasating neutrophils open vascular barrier and improve liposomes delivery to tumors. *ACS Nano* **13**, 12599–12612 (2019).
97. Ballou, B. et al. Sentinel lymph node imaging using quantum dots in mouse tumor models. *Bioconjug. Chem.* **18**, 389–396 (2007).
98. Valdés-Olmos, R. A. et al. Evaluation of mammary lymphoscintigraphy by a single intratumoral injection for sentinel node identification. *J. Nucl. Med.* **41**, 1500–1506 (2000).
99. Liang, C. et al. Tumor metastasis inhibition by imaging-guided photothermal therapy with single-walled carbon nanotubes. *Adv. Mater.* **26**, 5646–5652 (2014).
100. Liu, J. et al. Enhanced primary tumor penetration facilitates nanoparticle draining into lymph nodes after systemic injection for tumor metastasis inhibition. *ACS Nano* **13**, 8648–8658 (2019).
101. Qin, L. et al. A tumor-to-lymph procedure navigated versatile gel system for combinatorial therapy against tumor recurrence and metastasis. *Sci. Adv.* **6**, eabb3116 (2020).
102. Jiang, X. et al. Intratumoral administration of STING-activating nanovaccine enhances T cell immunotherapy. *J. Immunother. Cancer* **10**, e003960 (2022).
103. Kwong, B., Gai, S. A., Elkhader, J., Wittrup, K. D. & Irvine, D. J. Localized immunotherapy via liposome-anchored anti-CD137 + IL-2 prevents lethal toxicity and elicits local and systemic antitumor immunity. *Cancer Res.* **73**, 1547–1558 (2013).
104. Ludford, R. J. The vital staining of normal and malignant cells.-II. The staining of malignant tumours with trypan blue. *Proc. R. Soc. Lond. B* **104**, 493–512 (1929).
105. Lin, Z. P. et al. Macrophages actively transport nanoparticles in tumors after extravasation. *ACS Nano* **16**, 6080–6092 (2022).
This article reports that nanoparticle transport through the tumour occurs via cellular-based mechanisms, showing that perivascular macrophages sequester extravasated nanoparticles and transport them throughout the tumour.
106. Miller, M. A. et al. Tumour-associated macrophages act as a slow-release reservoir of nano-therapeutic Pt(IV) pro-drug. *Nat. Commun.* **6**, 8692 (2015).
107. Haber, T. et al. Specific targeting of ovarian tumor-associated macrophages by large, anionic nanoparticles. *Proc. Natl Acad. Sci.* **117**, 19737–19745 (2020).
108. Walkey, C. D., Olsen, J. B., Guo, H., Emili, A. & Chan, W. C. W. Nanoparticle size and surface chemistry determine serum protein adsorption and macrophage uptake. *J. Am. Chem. Soc.* **134**, 2139–2147 (2012).
109. Menard, J. A., Cerezo-Magaña, M. & Belting, M. Functional role of extracellular vesicles and lipoproteins in the tumour microenvironment. *Philos. Trans. R. Soc. B Biol. Sci.* **373**, 20160480 (2018).
110. Frankel, W. L. & Jin, M. Serosal surfaces, mucin pools, and deposits, Oh my: challenges in staging colorectal carcinoma. *Mod. Pathol.* **28**, S95–S108 (2015).
111. Timpl, R. Structure and biological activity of basement membrane proteins. *Eur. J. Biochem.* **180**, 487–502 (1989).
112. Yurchenco, P. D. Basement membranes: cell scaffoldings and signaling platforms. *Cold Spring Harb. Perspect. Biol.* **3**, a004911 (2011).
113. Kirkland, S. C. Type I collagen inhibits differentiation and promotes a stem cell-like phenotype in human colorectal carcinoma cells. *Br. J. Cancer* **101**, 320–326 (2009).
114. Medici, D. & Nawshad, A. Type I collagen promotes epithelial–mesenchymal transition through ILK-dependent activation of NF-κB and LEF-1. *Matrix Biol.* **29**, 161–165 (2010).
115. Januchowski, R. et al. Increased expression of several collagen genes is associated with drug resistance in ovarian cancer cell lines. *J. Cancer* **7**, 1295–1310 (2016).
116. Sherman-Baust, C. A. et al. Remodeling of the extracellular matrix through overexpression of collagen VI contributes to cisplatin resistance in ovarian cancer cells. *Cancer Cell* **3**, 377–386 (2003).
117. Yurchenco, P. D. & Ruben, G. C. Basement membrane structure in situ: evidence for lateral associations in the type IV collagen network. *J. Cell Biol.* **105**, 2559–2568 (1987).

118. Pluen, A. et al. Role of tumor–host interactions in interstitial diffusion of macromolecules: cranial vs. subcutaneous tumors. *Proc. Natl Acad. Sci.* **98**, 4628–4633 (2001).
119. Lieleg, O., Baumgärtel, R. M. & Bausch, A. R. Selective filtering of particles by the extracellular matrix: an electrostatic bandpass. *Biophys. J.* **97**, 1569–1577 (2009).
120. Stylianopoulos, T. et al. Diffusion of particles in the extracellular matrix: the effect of repulsive electrostatic interactions. *Biophys. J.* **99**, 1342–1349 (2010).
121. Dancy, J. G. et al. Non-specific binding and steric hindrance thresholds for penetration of particulate drug carriers within tumor tissue. *J. Control. Release* **238**, 139–148 (2016).
122. Sykes, E. A. et al. Tailoring nanoparticle designs to target cancer based on tumor pathophysiology. *Proc. Natl Acad. Sci.* **113**, E1142–E1151 (2016).
123. Wang, X. et al. Delivery of platinum(IV) drug to subcutaneous tumor and lung metastasis using bradykinin-potentiating peptide-decorated chitosan nanoparticles. *Biomaterials* **35**, 6439–6453 (2014).
124. Appiah, E. et al. Acid-responsive HPMA copolymer-bradykinin conjugate enhances tumor-targeted delivery of nanomedicine. *J. Control. Release* **337**, 546–556 (2021).
125. Gormley, A. J. et al. Guided delivery of polymer therapeutics using plasmonic photothermal therapy. *Nano Today* **7**, 158–167 (2012).
126. Zhen, Z. et al. Tumor vasculature targeted photodynamic therapy for enhanced delivery of nanoparticles. *ACS Nano* **8**, 6004–6013 (2014).
127. Sano, K., Nakajima, T., Choyke, P. L. & Kobayashi, H. Markedly enhanced permeability and retention effects induced by photo-immunotherapy of tumors. *ACS Nano* **7**, 717–724 (2013).
128. Liang, C. et al. Nanoparticle-mediated internal radioisotope therapy to locally increase the tumor vasculature permeability for synergistically improved cancer therapies. *Biomaterials* **197**, 368–379 (2019).
129. Ashton, J. R. et al. Dual-energy CT imaging of tumor liposome delivery after gold nanoparticle-augmented radiation therapy. *Theranostics* **8**, 1782–1797 (2018).
130. Feng, D. et al. Pathways of macromolecular extravasation across microvascular endothelium in response to VPF/VEGF and other vasoactive mediators. *Microcirculation* **6**, 23–44 (1999).
131. Dictor, M., Carlén, B., Bendsöe, N. & Flamholc, L. Ultrastructural development of Kaposi's sarcoma in relation to the dermal microvasculature. *Virchows Arch.* **419**, 35–43 (1991).
132. Orenstein, J. M. Ultrastructure of Kaposi sarcoma. *Ultrastruct. Pathol.* **32**, 211–220 (2008).
133. Jenny, B. et al. Expression and localization of VEGF-C and VEGFR-3 in glioblastomas and haemangioblastomas. *J. Pathol.* **209**, 34–43 (2006).
134. Walkey, C. D. & Chan, W. C. W. Understanding and controlling the interaction of nanomaterials with proteins in a physiological environment. *Chem. Soc. Rev.* **41**, 2780–2799 (2011).
135. Cedervall, T. et al. Understanding the nanoparticle–protein corona using methods to quantify exchange rates and affinities of proteins for nanoparticles. *Proc. Natl Acad. Sci.* **104**, 2050–2055 (2007).
136. Tenzer, S. et al. Rapid formation of plasma protein corona critically affects nanoparticle pathophysiology. *Nat. Nanotechnol.* **8**, 772–781 (2013).
137. Lazarovits, J. et al. Supervised learning and mass spectrometry predicts the in vivo fate of nanomaterials. *ACS Nano* **13**, 8023–8034 (2019).
138. Yan, Y. et al. Differential roles of the protein corona in the cellular uptake of nanoporous polymer particles by monocyte and macrophage cell lines. *ACS Nano* **7**, 10960–10970 (2013).
139. Ngo, W. et al. Identifying cell receptors for the nanoparticle protein corona using genome screens. *Nat. Chem. Biol.* **18**, 1023–1031 (2022).
140. Goncalves, A. et al. Micellar lipid composition affects micelle interaction with class B scavenger receptor extracellular loops. *J. Lipid Res.* **56**, 1123–1133 (2015).
141. Hatami, E. et al. Mannose-decorated hybrid nanoparticles for enhanced macrophage targeting. *Biochem. Biophys. Rep.* **17**, 197–207 (2019).
142. Schnitzer, J. E. gp60 is an albumin-binding glycoprotein expressed by continuous endothelium involved in albumin transcytosis. *Am. J. Physiol. Heart C.* **262**, H246–H254 (1992).
143. Ouyang, B. et al. The dose threshold for nanoparticle tumour delivery. *Nat. Mater.* **19**, 1362–1371 (2020).
144. Poon, W. et al. Elimination pathways of nanoparticles. *ACS Nano* **13**, 5785–5798 (2019).
145. Tsoi, K. M. et al. Mechanism of hard-nanomaterial clearance by the liver. *Nat. Mater.* **15**, 1212–1221 (2016).
146. Choi, H. S. et al. Renal clearance of quantum dots. *Nat. Biotechnol.* **25**, 1165–1170 (2007).
147. Chauhan, V. P. et al. Normalization of tumour blood vessels improves the delivery of nanomedicines in a size-dependent manner. *Nat. Nanotechnol.* **7**, 383 (2012).
148. Stylianopoulos, T., Munn, L. L. & Jain, R. K. Reengineering the physical microenvironment of tumors to improve drug delivery and efficacy: from mathematical modeling to bench to bedside. *Trends Cancer* **4**, 292–319 (2018).
149. Zhang, Y.-N. et al. Nanoparticle size influences antigen retention and presentation in lymph node follicles for humoral immunity. *Nano Lett.* **19**, 7226–7235 (2019).
150. Zhang, Y.-N., Poon, W., Sefton, E. & Chan, W. C. W. Suppressing subcapsular sinus macrophages enhances transport of nanovaccines to lymph node follicles for robust humoral immunity. *ACS Nano* **14**, 9478–9490 (2020).
151. Nakamura, T. et al. The effect of size and charge of lipid nanoparticles prepared by microfluidic mixing on their lymph node transitivity and distribution. *Mol. Pharm.* **17**, 944–953 (2020).
152. Sykes, E. A., Chen, J., Zheng, G. & Chan, W. C. W. Investigating the impact of nanoparticle size on active and passive tumor targeting efficiency. *ACS Nano* **8**, 5696–5706 (2014).
153. He, C., Hu, Y., Yin, L., Tang, C. & Yin, C. Effects of particle size and surface charge on cellular uptake and biodistribution of polymeric nanoparticles. *Biomaterials* **31**, 3657–3666 (2010).
154. Liu, X. et al. Mixed-charge nanoparticles for long circulation, low reticuloendothelial system clearance, and high tumor accumulation. *Adv. Healthc. Mater.* **3**, 1439–1447 (2014).
155. Wang, J. et al. Size- and surface chemistry-dependent pharmacokinetics and tumor accumulation of engineered gold nanoparticles after intravenous administration. *Metallomics* **7**, 516–524 (2015).
156. Han, X. et al. Stealth CD44-targeted hyaluronic acid supramolecular nanoassemblies for doxorubicin delivery: probing the effect of uncovalent pegylation degree on cellular uptake and blood long circulation. *J. Control. Release* **197**, 29–40 (2015).
157. Al-Jamal, W. T. et al. Tumor targeting of functionalized quantum dot–liposome hybrids by intravenous administration. *Mol. Pharm.* **6**, 520–530 (2009).
158. Goel, S. et al. VEGF121-conjugated mesoporous silica nanoparticle: a tumor targeted drug delivery system. *ACS Appl. Mater. Inter.* **6**, 21677–21685 (2014).
159. Perrault, S. D., Walkey, C., Jennings, T., Fischer, H. C. & Chan, W. C. W. Mediating tumor targeting efficiency of nanoparticles through design. *Nano Lett.* **9**, 1909–1915 (2009).
160. Arnida, Janát-Amsbury, M. M., Ray, A., Peterson, C. M. & Ghandehari, H. Geometry and surface characteristics of gold nanoparticles influence their biodistribution and uptake by macrophages.*Eur. J. Pharm. Biopharm.* **77**, 417–423 (2011).
161. Mitchell, M. J. et al. Engineering precision nanoparticles for drug delivery. *Nat. Rev. Drug Discov.* **20**, 101–124 (2021).
162. Blanco, E., Shen, H. & Ferrari, M. Principles of nanoparticle design for overcoming biological barriers to drug delivery. *Nat. Biotechnol.* **33**, 941–951 (2015).
163. Tylawsky, D. E. et al. P-selectin-targeted nanocarriers induce active crossing of the blood–brain barrier via caveolin-1-dependent transcytosis. *Nat. Mater.* **22**, 391–399 (2023).
164. Zhou, Q. et al. Enzyme-activatable polymer–drug conjugate augments tumour penetration and treatment efficacy. *Nat. Nanotechnol.* **14**, 799–809 (2019).
165. Chen, S. et al. Enhanced tumour penetration and prolonged circulation in blood of polyzwitterion–drug conjugates with cell-membrane affinity. *Nat. Biomed. Eng.* **5**, 1019–1037 (2021).
166. Wang, Q. et al. Breaking through the basement membrane barrier to improve nanotherapeutic delivery to tumours.*Nat. Nanotechnol.* **19**, 95–105 (2024).
167. Matsumoto, Y. et al. Vascular bursts enhance permeability of tumour blood vessels and improve nanoparticle delivery. *Nat. Nanotechnol.* **11**, 533–538 (2016).
168. Miller, M. A. et al. Radiation therapy primes tumors for nanotherapeutic delivery via macrophage-mediated vascular bursts.*Sci. Transl. Med.* **9**, eaal0225 (2017).
169. Foroozandeh, P. & Aziz, A. A. Insight into cellular uptake and intracellular trafficking of nanoparticles. *Nanoscale Res. Lett.* **13**, 339 (2018).
170. Wolfram, J. et al. A chloroquine-induced macrophage-preconditioning strategy for improved nanodelivery. *Sci. Rep.* **7**, 13738 (2017).

Acknowledgements

W.C.W.C. acknowledges Collaborative Health Research Program Grant CPG-146468, Canadian Institute of Health Research Grants FDN159932 and MOP-1301431, and Canadian Research Chairs Program Grant 950-223824. We thank NSERC (W.N. and S.M.M.), Ontario Graduate Scholarship (P.M. and S.M.M.), the Cecil Yip Award (W.N.), the Walter C. Sumner Foundation (P.M.), Vanier Canada Graduate Scholarship (S.M.M.), Jennifer Dorrington Award (S.M.M.) and the Wildcat Foundation (W.N.) for student fellowships and scholarships. We thank J. Rothschild and A. Zilman for commenting on the manuscript.

Author contributions

L.N.M.N., S.S. and W.C.W.C. outlined the initial manuscript format. L.N.M.N., W.N. Z.P.L., S.S., P.M. and W.C.W.C. performed the literature search and wrote the initial manuscript draft. L.N.M.N., W.N., Z.P.L. and S.M.M. designed the figures. All authors participated in the writing and editing of the manuscript.

Competing interests

The authors declare the following competing interests: W.C.W.C. is a co-founder of Luna Nanotech and consults for Foresite Capital, the Cystic Fibrosis Foundation, METiS Therapeutics, Moderna and Merck. L.N.M.N., W.N., Z.P.L., S.S., P.M. and S.M.M. declare no competing interests.

Additional information

Peer review information *Nature Reviews Bioengineering* thanks Twan Lammers, Michael Mitchell and the other, anonymous, reviewer(s) for their contribution to the peer review of this work.

Related links

The fundamental processes involved in or responsible for an action, reaction, or other natural phenomenon: https://www.merriam-webster.com/dictionary/mechanism